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International Patent Application PCT/EP 98/04510

BioteCon GmbH

Patent claims 1 to 33
according to Art. 34 Chapter II PCT

1. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides.
2. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides.
3. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one

nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from

- (i) nucleotide position 54 to 83 of SEQ ID NO 1, or
- (ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
- (iii) the sequences complementary to (i) or (ii).

4. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid amplification and/or nucleic acid hybridisation methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected .

5. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid hybridisation and/or

nucleic acid amplification methods under reaction conditions known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in

- (i) the region 54 to 83 of SEQ ID NO 1, or
- (ii) the region 100 to 166 of SEQ ID NO 1, or
- (iii) the sequence that is complementary to the region according to (i) or (ii)

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

6. Kit according to claim 5, **characterised** by a nucleic acid molecule that has the SEQ ID NO 1 or its complementary sequence.

7. Kit according to claim 6, **characterised** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

- (i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or
- (ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or
- (iii) a sequence that is complementary to a sequence according to (i) or (ii).

8. Kit according to claim 6, **characterised** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

- (i) SEQ ID NO 2, or
- (ii) SEQ ID NO 3, or

- (iii) SEQ ID NO 4, or
- (iv) the sequences complementary to (i), (ii) and (iii), respectively.

Claim 1

9. Kit according to any one of the preceding claims, **characterised** by a nucleic acid molecule that differs from a nucleic acid molecule according to any one of the preceding claims but that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

- (i) is identical to the nucleic acid molecule according to any one of the preceding claims, or
- (ii) corresponds in 9 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or
- (iii) corresponds in 8 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or
- (iv) is at least 90% homologous to the nucleic acid molecule according to any one of the preceding claims.

10. Kit according to claim 9, **characterised** in that the nucleic acid molecule is from 10 to 250, preferably from 15 to 30, nucleotides long, especially characterised in that it is the nucleic acid molecule having the sequence SEQ ID NO 5.

Claim 1

11. Kit according to any one of the preceding claims, **characterised** in that the nucleic acid molecule is single-stranded or double-stranded.

Claim 1

12. Kit according to any one of the preceding claims, **characterised** in that the nucleic acid molecule is present

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(i) as DNA, or

(ii) as RNA corresponding to (i), or

(iii) as PNA,

the nucleic acid molecule, where appropriate, being modified in a manner known *per se* for analytical detection methods, especially methods based on hybridisation and/or amplification.

13. Kit according to claim 12, **characterised** in that the nucleic acid molecule is modified by the replacement of up to 10% of the nucleotides, especially 1 or 2 nucleotides, by analogous components known *per se* for probes and/or primers, especially by nucleotides that do not occur naturally in bacteria.

14. Kit according to claim 12 or claim 13, **characterised** in that the nucleic acid molecule is modified or labelled or is additionally modified or labelled in that it comprises, in a manner known *per se* for analytical detection methods, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially an enzymatic reaction, especially using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes, or it comprises, in a manner known *per se* for analytical detection methods, groups that have been modified or that modify in some other manner.

15. Use of a kit according to any one of the preceding claims for the detection of the presence or absence of

claim 1

bacteria belonging to a group of bacteria of the genus *Staphylococcus*.

16. Use according to claim 15, **characterised** in that the group of bacteria of the genus *Staphylococcus* comprises various strains of *Staphylococcus aureus*.

17. Use according to claim 16, **characterised** in that the group of bacteria of the genus *Staphylococcus* comprises exclusively *Staphylococcus aureus* strains.

Claim 15

18. Use according to any one of claims 15 to 17, **characterised** in that nucleic acid hybridisation and/or nucleic acid amplification is carried out.

19. Use according to claim 18, **characterised** in that a polymerase chain reaction is carried out as nucleic acid amplification.

Claim 15

20. Use according to any one of claims 15 to 19, **characterised** in that the detection is carried out by distinguishing between the bacteria to be detected and bacteria that are not to be detected on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to any one of claims 1 to 14.

21. Use according to claim 20, **characterised** in that the distinction is made on the basis of differences in the region of a nucleic acid molecule according to claim 6.

22. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

23. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

24. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from
(i) nucleotide position 54 to 83 of SEQ ID NO 1, or
(ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
(iii) the sequences complementary to (i) or (ii),
excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

25. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, **characterised** in that it makes it possible by means of nucleic acid hybridisation

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and/or nucleic acid amplification methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

26. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, **characterised** in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under reaction conditions known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in
(i) the region 54 to 83 of SEQ ID NO 1, or
(ii) the region 100 to 166 of SEQ ID NO 1, or
(iii) the sequence that is complementary to (i) or (ii), in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

27. Nucleic acid molecule, **characterised** in that it has the SEQ ID NO 1 or its complementary sequence.

28. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

- (i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or
- (ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or
- (iii) a sequence that is complementary to a sequence according to (i) or (ii).

29. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

- (i) SEQ ID NO 3, or
- (ii) SEQ ID NO 4, or
- (iii) the sequences complementary to (i) and (ii), respectively.

30. Nucleic acid molecule, **characterised** in that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

- (i) it is identical to a nucleic acid molecule according to ~~any one of claims 22 to 29~~ ^{Claim 22}, or
- (ii) it corresponds in 9 out of 10 successive nucleotides to a nucleic acid molecule according to ~~any one of claims 22 to 29~~ ^{Claim 22}, or
- (iii) it corresponds in 8 out of 10 successive nucleotides to a nucleic acid molecule according to ~~any one of claims 22 to 29~~ ^{Claim 22}, or
- (iv) it is at least 90% homologous to a nucleic acid molecule according to ~~any one of claims 22 to 29~~ ^{Claim 22}.

31. Nucleic acid molecule, **characterised** in that it has the SEQ ID NO 5 or its complementary sequence.

A 32. Nucleic acid molecule according to any one of claims 22 to 30, **characterised** in that it is from 10 to 250, preferably from 15 to 30, nucleotides long.

A 33. Nucleic acid molecule according to any one of claims 22 to 32, **characterised** in that the nucleic acid molecule is single-stranded or double-stranded.

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